

## IN THE EUROPEAN PATENT OFFICE

In The Matter of PCT International Patent Application:

Applicant : University of Guelph  
Owner : University of Guelph  
PCT Appln. No. : PCT/CA2003/000964  
PCT Filing Date : June 27, 2003  
Title : Harvest - Inducible Genes From Alfafa (Medicago Sativa)  
And Methods Of Use Thereof  
Our File : 08892370WO  
Date : August 27, 2004

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European Patent Office  
Erhardstrasse 27  
D-80331 Munich  
GERMANY

### Response to Written Opinion Amendment under Article 34 of the PCT

Dear Sirs:

Pursuant to the Written Opinion dated May 28, 2004, please amend the above-identified application as follows:

#### IN THE CLAIMS

Claim 7: Amend.

Claims 1-6: Cancel.

#### REMARKS

Original claims 1-6 have been cancelled without prejudice or disclaimer, and Applicant reserves the right to reintroduce these claims at a latter date. As a result, all subsequent claims and their dependencies have been renumbered accordingly. New claims 1-20 remain pending in this application.

Claim 1 (old claim 7) has been amended to recite that each of the sequences defined have harvest-inducible regulatory activity. Support for this amendment may be found for example on page 16, second full paragraph.

Applicant submits that while not agreeing with Examiner's objection to claims 1-6 under Article 33(2)(3) PCT as lacking novelty and inventive step, by canceling claims 1-6, this objection is rendered moot.

Examiner objected to claim 7-26, alleging that it is not apparent that a fragment of a harvest-inducible regulatory element would provide a solution to the technical problem. Applicant respectfully disagrees.

Claim 1 (old claim 7), as amended, recites that SEQ ID NO: 4-6, fragments of SEQ ID NO: 4-6, or sequences and fragments that hybridize to SEQ ID NO: 4-6, have a specified utility, that of harvest-inducible regulatory activity as defined on page 16 (2<sup>nd</sup> paragraph) of the specification. Applicant submits that the subject matter of new claim 1 and dependant claims 2-20 (old claims 8-26) is not disclosed or suggested in any of cited prior art, and that the claims meet the requirements of Article 33(3) PCT.

We trust that, with the above amendments, this application is now in a form acceptable for favourable consideration by the Examiner

Respectfully submitted,

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**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OF PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A regulatory element selected from the group consisting of:

i) SEQ ID NO:4, a complement thereof, a fragment of SEQ ID NO:4, a complement of a fragment of SEQ ID NO:4, a nucleic acid that hybridizes to SEQ ID NO:4 under stringent hybridization conditions, a nucleic acid that hybridizes to a complement of SEQ ID NO:4 under stringent hybridization conditions, a nucleic acid that hybridizes to a fragment of SEQ ID NO:4 under stringent hybridization conditions, or a nucleic acid that hybridizes to a complement of fragment of SEQ ID NO:4 under stringent hybridization conditions, having harvest-inducible regulatory activity;

ii) SEQ ID NO:5, a complement thereof, a fragment of SEQ ID NO:5, a complement of a fragment of SEQ ID NO:5, a nucleic acid that hybridizes to SEQ ID NO:5 under stringent hybridization conditions, a nucleic acid that hybridizes to a complement of SEQ ID NO:5 under stringent hybridization conditions, a nucleic acid that hybridizes to a fragment of SEQ ID NO:5 under stringent hybridization conditions, or a nucleic acid that hybridizes to a complement of fragment of SEQ ID NO:5 under stringent hybridization conditions, having harvest-inducible regulatory activity; and

iii) SEQ ID NO:6, a complement thereof, a fragment of SEQ ID NO:6, a complement of a fragment of SEQ ID NO:6, a nucleic acid that hybridizes to SEQ ID NO:6 under stringent hybridization conditions, a nucleic acid that hybridizes to a complement of SEQ ID NO:6 under stringent hybridization conditions, a nucleic acid that hybridizes to a fragment of SEQ ID NO:6 under stringent hybridization conditions, or a nucleic acid that hybridizes to a complement of fragment of SEQ ID NO:6 under stringent hybridization conditions, having harvest-inducible regulatory activity,

the stringent hybridization conditions comprising, hybridization overnight (12-24 hrs) at 42°C in the presence of 50% formamide, followed by washing, or 5X SSC at about 65°C for about 12 to about 24 hours, followed by washing in 0.1X SSC at 65°C for about one hour, wherein the regulatory element exhibits harvest-inducible activity.

2. A construct comprising said harvest-inducible regulatory element of claim 1, operably linked with a heterologous coding sequence of interest and a terminator region.

3. A construct comprising a heterologous coding sequence operably linked to the harvest-inducible regulatory element of claim 1, the harvest-inducible regulatory element further comprising a nucleotide sequence encoding a harvest-inducible protein or fragment thereof.

4. A vector comprising the DNA construct of claim 2.

5. A vector comprising the DNA construct of claim 3.

6. A plant, plant tissue, plant seed, plant cell, or progeny therefrom, comprising the construct of claim 2.

7. A plant, plant tissue, plant seed, plant cell, or progeny therefrom, comprising the construct of claim 3.

8. A method for production of a heterologous protein in a plant comprising:

- i) providing a plant transformed with the construct of claim 2;
- ii) growing the transformed plant; and
- iii) harvesting the transformed plant thereby inducing expression of the heterologous protein.

9. The method of claim 8, wherein the step of harvesting (step iii) is followed by:

- iv) isolating the heterologous protein from the transformed plant.

10. The method of claim 9, wherein the step of isolating (step iv)) is followed by a step of purification of the heterologous protein.

11. A method for production of a heterologous protein in a plant comprising,

- i) providing a plant transformed with the construct of claim 3;
- ii) growing the transformed plant; and
- iii) harvesting the transformed plant to induce expression of the heterologous protein.

12. The method of claim 11, wherein the step of harvesting (step iii) is followed by:  
iv) isolating the heterologous protein from the transformed plant.
13. The method of claim 12, wherein the step of isolating (step iv)) is followed by a step of purification of the heterologous protein.
14. A method for production of a heterologous protein in a plant comprising:  
i) providing a plant expressing the construct of claim 2;  
ii) growing the plant; and  
iii) harvesting the plant thereby inducing expression of the heterologous protein.
15. A method for production of a heterologous protein in a plant comprising,  
i) providing plant expressing the construct of claim 3;  
ii) growing transformed plant; and  
iii) harvesting the plant to induce expression of the heterologous protein.
16. A harvest inducible regulatory element according to claim 1, wherein the harvest inducible regulatory element is SEQ ID NO:4.
17. A harvest inducible regulatory element according to claim 1, wherein the harvest inducible regulatory element is SEQ ID NO:5.
18. A harvest inducible regulatory element according to claim 1, wherein the harvest inducible regulatory element is SEQ ID NO:6.
19. The plant, plant tissue, plant seed, plant cell, or progeny therefrom according to claim 6, wherein the plant, plant tissue, plant seed, plant cell, or progeny therefrom is selected from the group consisting of potato, tomato, canola, corn, soybean, alfalfa, pea, lentil, other forage legumes such as clover, trefoil, forage grasses such as timothy, ryegrass, brome grass, fescue or other cereal grasses used for forage such as barley, wheat, sudan grass, sorgham.
20. The plant, plant tissue, plant seed, plant cell, or progeny therefrom according to claim 7, wherein the plant, plant tissue, plant seed, plant cell, or progeny therefrom is selected from the group consisting of potato, tomato, canola, corn, soybean, alfalfa, pea, lentil, other forage

legumes such as clover, trefoil, forage grasses such as timothy, ryegrass, brome grass, fescue or other cereal grasses used for forage such as barley, wheat, sudan grass, sorgham.